Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 5| Issue 1|JAN-MAR|2015|130-137

Research Article
Biological Sciences

QUANTITATION OF EMBELIN FROM PLANT *EMBELIA DRUPACEA* (DENNST) M. R & S. M. ALMEIDA BY DEVELOPMENT AND VALIDATION OF H.P.T.L.C. METHOD

V. P. Sathe [1*] & A.P. Dixit [2]

[*1] Botany department, Kirti M. Doongursee college, Dadar, Mumbai, Maharashtra, India.

[2] Botany department, DSPM'S K.V.Pendharkar college. Dombivali, Thane, Maharashtra, India.

University of Mumbai.

*Corresponding Author Email: vpsathe14@gmail.com

ABSTRACT

Fruits of Embelia drupacea known locally as Ambat are traditionally employed as laxative, antiseptic and antihelmintic and could be use as substitute for Ayurvedic drug 'Vidanga' Embelia ribes Burm F. 'Vidanga' contains as many as 35 bioactive compounds of which Embelin is most important one. Quntitation of embelin from fruits of E. drupacea also belonging to family Myrsinaceae by development and validation of H.P.T.L.C method has been demonstrated in this communication. For estimation of Embelin from E. drupacea fruits a selective, precise and accurate High Performance Thin Layer Chromatography (H.P.T.L.C) method has been developed. The method developed involved TLC aluminium plate (20×10 with thickness 250 µm) precoated with Silica gel 60 F_{254} as stationary phase with solvent system Chloroform: Ethyl acetate: Formic acid (5:4:1 v/v/v). Densitometric analysis was carried out in the absorbance mode at 348 nm using Camag TLC Scanner – IV. The developed HPTLC method showed good regression (r^2 = 0.99795 ± 0.0020) and the recovery of Embelin was in the range of 97% - 113%. The limit of detection and limit of quantitation were found to be 0.69 ng / spot and 2.11 ng / spot respectively. The method was validated for precision, recovery, limit of detection, limit of quantitation and linearity. H.P.T.L.C. quantitation was done at multilevel (for all concentrations). The developed method was found to be simple, precise and accurate and could be used for the quality control of the raw material as well as formulations.

KEY WORDS

Ambat, Embelia drupacea, Embelin, HPTLC, Validation.

INTRODUCTION

Embelia ribes Burm F. and *E. drupacea* of Myrsinaceae are woody climbers growing in semi-evergreen to deciduous forests up to an altitude of 1600 m. throughout India [1]. Known in Ayurveda as 'Vidanga' [6] and 'Ambat' [9] respectively, 'Vidanga' is used in as many as 75 Ayurvedic preparations. Major organ used is fruit that is claimed to be having anthelmintic, carminative, hypoglycemic and antifertility properties. While fruits of 'Ambat' are used as laxative, antiseptic and antihelmintic and root bark in toothache [3]. 'Vidanga' contains as many as 35 bioactive compounds of which quinones, Embelin and Vilangin are most important ones [8]. Embelin in powdered drug of *Embelia ribes* Burm F. is reported to be 4.21 to 4.65 % [6]. Presence of Embelin has also been reported in *Embelia basaal* (Raem and Schalt) DC. 1.6% [4] and a quinone i.e kirtiquinone similar to embelin in *Maesa indica* (Roxb.) DC.[2]. Dried fruits of *E. ribes*. Burm. F are a high volume (>500 MT /

year), top traded botanical drug employed not only by Ayurveda but also by other Indian traditional systems of medicine like Siddha & Unani [5]. Because of excessive exploitation of reproductive structures from the natural populations and as the natural regeneration has been reported to be very poor, the species is also red listed [4] and has been considered to be one of the 32 medicinal plant species identified by Medical Board, Govt. of India, New Delhi as being important for large scale cultivation. However attempts of artificial regeneration of *E. ribes*. Burm. F have still not succeeded due to high levels of seed sterility, poor natural regeneration , slow rate of germination of viable seeds and poor rooting response of stem cuttings. Unknown propagation techniques has resulted in the lack of quality planting material for promoting cultivation [4]

The sporadic occurrence of the species in its natural distributional range of Western Ghats, Eastern Himalayas and North East India indicates that the high volumes traded (>500MT / year) cannot be contributed by *E. ribes* alone, an authentic source of 'Vidanga' by official pharmacopeia. There is possibility of fruits of *Embelia drupacea* [1] being used as substitutes for 'Vidanga' as it belongs to same genus of family Myrsinaceae. Since standardization of any drug initiates with the purity of raw source used, it becomes important to identify and quantify the major constituent embelin. Embelin (derivative of benzoquinone) is found to be the active principle of *Embelia ribes*. Chemical structure of Embelin has a long chain (undecyl) as a substituent, which confers solubility in the non polar phase and cell permeability. The adjacent quinine and hydroquinone groups on embelin form intramolecular hydrogen bonds. Embelin is insoluble in water and soluble in alcohol, chloroform and benzene. Development and Validation of HPTLC method for embelin was reported on marketed formulations [10]. Under this back ground the present communication first time reports quantitation of Embelin from fruits of *Embelia drupacea* collected from natural source with development and validation of H.P.T.L.C. method for estimation of Embelin to substantiate its use as substitute for *Embelia ribes*.

MATERIAL AND METHOD [10]

Instrument-

Analysis was performed on 20 × 10 cm. 250 µm thick precoated with Silica gel 60 F_{254} TLC plates (E. MERCK KG). Samples were applied to the plates by means of CAMAG Linomet 5 automatic sample spotter with the aid of Hamilton 100 µl syringe. The TLC plates were developed in flat bottom twin trough Chamber. Detection (densitometry) was performed with a CAMAG TLC Scanner LINKED TO WinCAT S software.

Material

Mature fruits of *Embelia drupacea* were collected from Mahabaleshwar forest in the month of May 2014 and dried in oven at 37^0 c (Fig.1). The collected plant and fruits of *Embelia drupacea* were authenticated at Botanical survey of India, Pune and deposited as collection no. VPS02 at BSI Pune. All the chemicals used were of A.R. grade obtained from Merck Chemicals, India.

Preparation of Extracts of sample

The dried fruit powder was sieved, macerated with methanol and sonicated for 15 min. and centrifuged. The filtrate was used for HPTLC analysis.

Preparation of Standard solution

2 mg of Embelin (≥ 98% HPLC, powder make: Sigma) was dissolved in 2 ml of methanol and sonicated for 2 min. Then 1 ml. of sonicated methanol was taken and diluted to 10 ml. to get 0.1 mg / ml. Concentration of application from 100-500 ng/spot.

Fig.1- *Embelia drupacea* fruits

Preparation of Sample solution

500 mg of dried fruit powder was dissolved in 5 ml of methanol and sonicated for 15 min. After sonication the solution was centrifuged and filtrate was taken. Appropriate aliquots from these stock solutions were further diluted with same solvent to obtain 75 µg/ ml spot of embelin.

Preparation of Mobile phase

The mobile phase i.e Chloroform: ethyl acetate: formic acid in the proportion of 5:4:1 v/v/v was prepared in CAMAG twin – trough chamber by mixing and chamber was saturated for 10 minutes [10].

Validation

The developed method has been validated in terms of linearity, precision, specificity, robustness and accuracy as per International conference on Harmonization ICH Tripartite guidelines [7]. Quantitative analysis of embelin in sample *Embelia drupacea* was done by multilevel analysis (for all concentrations).

Calibration

Analysis was performed on 20 × 10 cm precoated Silica gel 60 F_{254} TLC plate with 250 µm thickness. The linear ascending development was carried out in glass twin-trough chamber (20 × 10 cm). Standard stock solutions of Embelin 0.1 mg / ml in methanol were applied on TLC plates in concentration 100-500 ng /spot using Camag Linomet 5 sample applicator under nitrogen stream. The TLC plate was dried in air with the help of drier and densitometric scan was performed at 348 nm with Camag TLC Scanner IV. Each amount was analyzed five times and peak areas were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for embelin.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae [10].

LOD = 3.3 (SD) / S

LOQ = 10 (SD) / S

Where, SD = Standard Deviation of response

S = Avg. of the slope of the calibration curve.

Precision

Precision of the method was verified by repeatability (intra-day) and intermediate (inter-day) studies. Intra-day and inter-day precisions were performed by analytical sample solution of 300 ng / spot of embelin three times on the same day and three different days respectively. Measurement of peak area for active compound was expressed in terms of % Relative Standard Deviation (% RSD).

Accuracy

Accuracy of method was tested by carrying out recovery studies at different spiked level by standard addition method. Standard embelin solution was added at three different levels (80, 100, 120 %). At each level three determinations

were performed and results were calculated by the difference between the Spiked and un- spiked sample analyzed under the same conditions.

Robustness

To determine the robustness of the developed method, few parameters such as variation in development distance (82 mm and 78 mm) were carried out.

Quantitative analysis of Embelin in *Embelia drupacea*

For assay of mature dried fruit powder, extracts were prepared as mentioned in the above section and subjected to optimized HPTLC conditions.

RESULT AND DISCUSSIONS

Method development and optimization

Initially many solvent systems were tried. Different system parameters such as composition of mobile phase, method of sample preparation, detection wavelength concentration of sample, amount of sample application were modified to obtain well resolved densitogram.

A solvent system that would give dense and compact spots with appropriate RF value was desired for quantitation of Embelin in collected sample of *Embelia drupacea.* Various solvent systems like Toluene : ethyl acetate: acetic acid (5:4:1), Chloroform : ethyl acetate: formic acid(5: 4: 0.5) , Chloroform : ethyl acetate: formic acid(5: 4: 1) in different proportions were tried. It was found that in the Toluene : ethyl acetate: formic acid (5:5:0.5 v/v/v),and Chloroform : ethyl acetate: formic acid(5: 4: 0.5) the peaks were not symmetrical and sharp but in Chloroform : ethyl acetate: formic acid(5: 4: 1 v/v/v) could show a sharp and symmetrical peak with Rf 0.58. [5]

HPTLC fingerprint analysis of *Embelia drupacea* (fig.2.) showed characteristic peak of Embelin (fig.3,4) at Rf value of 0.58. To obtain the fingerprints of authenticated sample, densitogram of samples were overlaid with the densitogram of isolated chemical marker Embelin as shown in (fig.5) which clearly indicated common peak in the sample. It is evident that sample shows peaks at same Rf value as that of Embelin (0.58) and hence can be said to contain same chemical component. Therefore *Embelia drupacea* can be used as substitute for *Embelia ribes* Burm F. in preparation of different ayurvedic medicines. For this however different pharmacognostic studies have to be carried out.

Fig.2. HPTLC finger print of Embelin at wavelength 366 nm.- Track 1,2 *E. drupacea* andTrack-3 Standard Embelin.

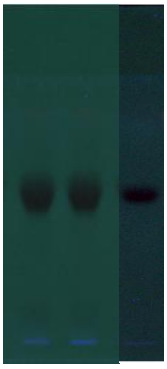


Fig.3. Chromatogram of Standard Embelin

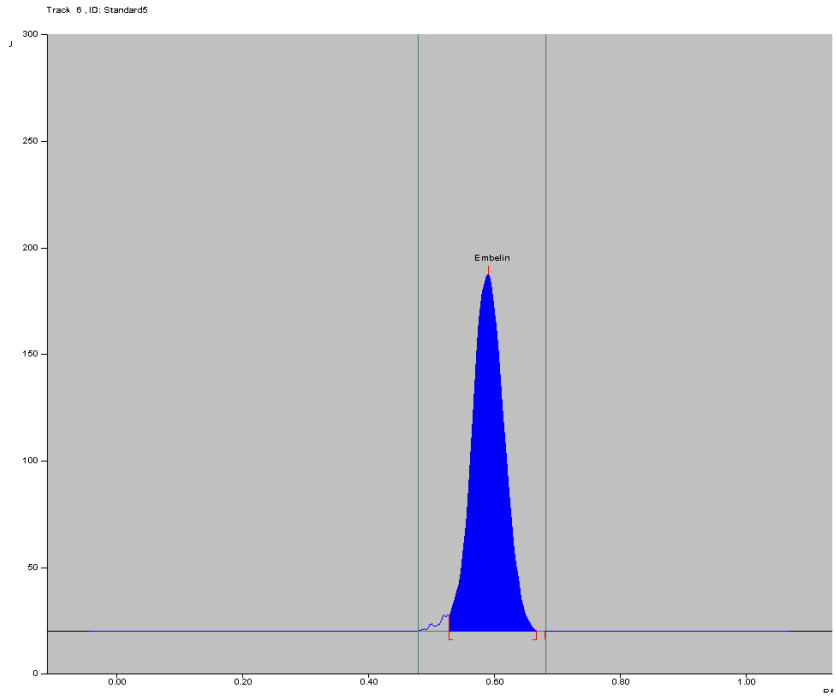


Fig.4. Chromatogram of Embelin in *E. drupacea*

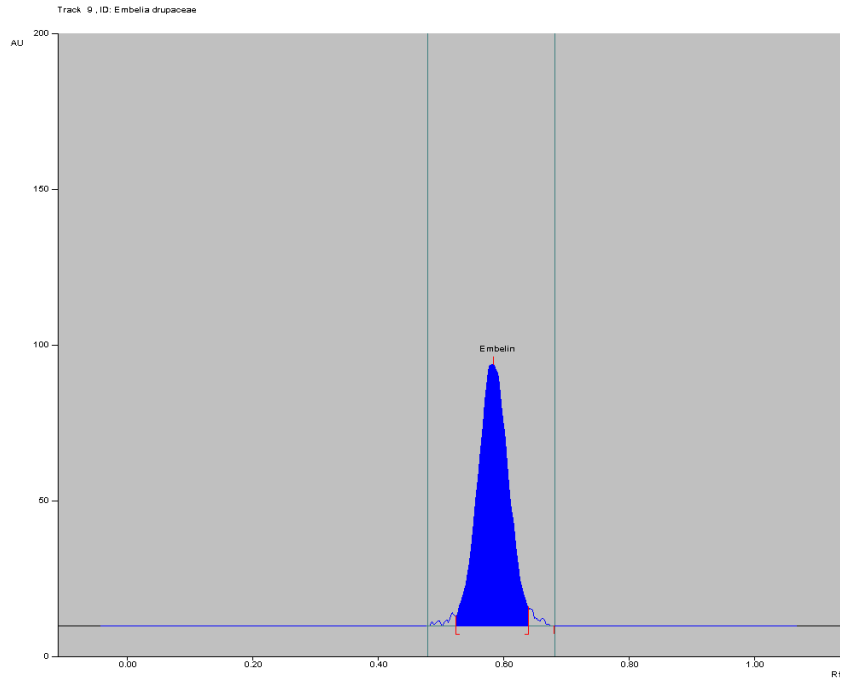


Fig.5. Densitogram - 3D View of Embelin in Standard and sample *E. drupacea.* Track 1-7 Standard Embelin, Track 8-10 *E. drupacea*.

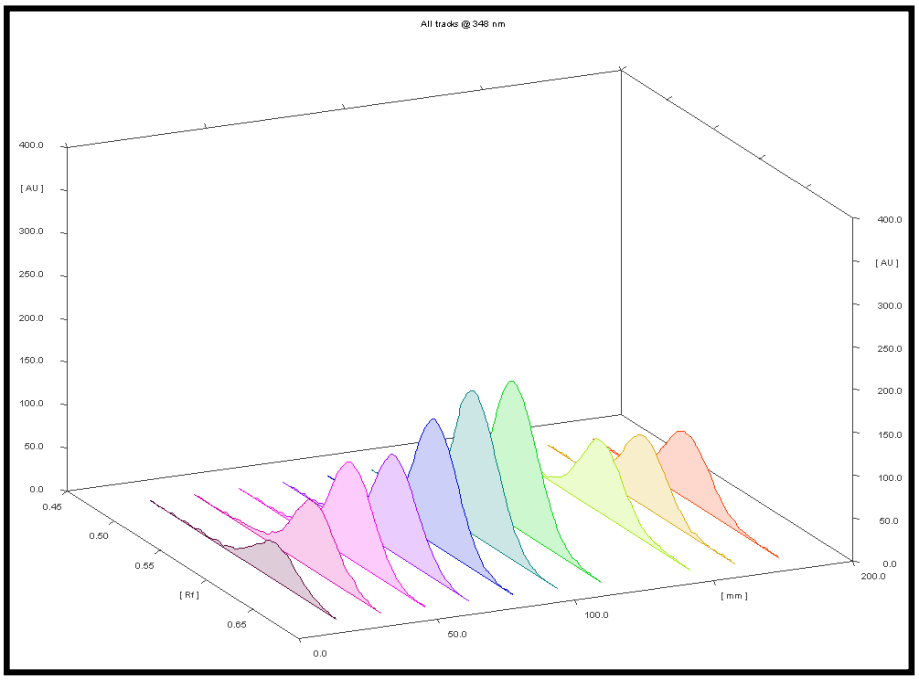
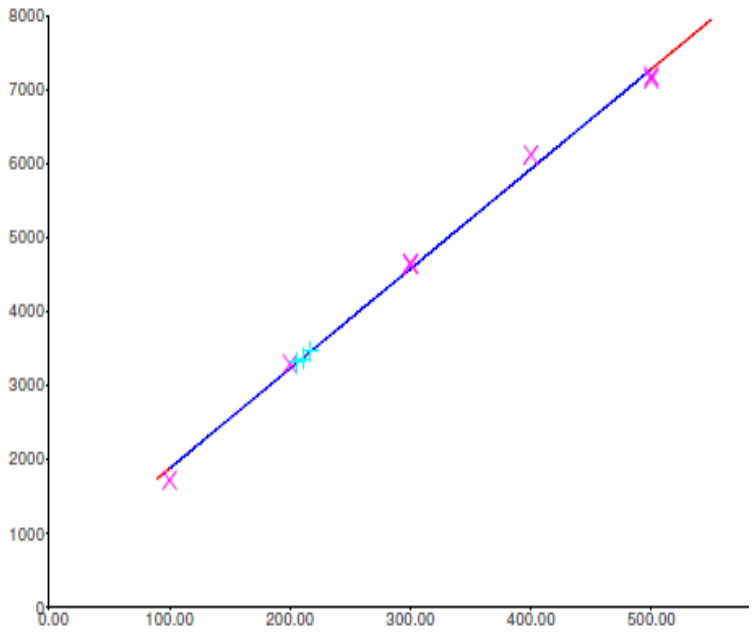


Fig.6. Linearity graph for Embelin- Average peak area versus concentrations



Validation of method

Linearity plots and detection limits

The linearity for Embelin was established by plotting the peak area versus concentration (fig. 6). The linearity of calibration curves was verified by correlation study and the correlation coefficients were found to be r^2 = 0.99795. The

LOD and LOQ for Embelin were found to be 0.69 ng / spot and 2.11 ng / spot respectively. (Table no. 1)

Table no. 1 – Calibration curves, limit of detection and limit of Quantitation of Embelin

Linear range *	Correlation coefficient	LOD	LOQ
100 – 500 ng/ spot	0.99795	0.69 ng/spot	2.11 ng / spot

*Results are mean of five determinations.

Precision, accuracy and robustness

Five tracks of same volume applied by linomet 5 and analyzed by scanner IV, coefficient of variation found 1.469% which is under acceptable limit (acceptable limit is 2%). % RSD value for inter-day and intra-day precision based on peak measurement was found to be less than 2 (n = 5) indicating that the methods were found to be precise. The average recoveries of the embelin were in the range of 97% - 113%. Satisfactory recoveries with small % relative standard deviations (less than 2) were obtained that indicates the accuracy of the method (Table No. 2). The robustness study revealed that there were no significant differences in peak areas. Retention time and Rf values after small, deliberate variation of the analytical condition evaluated the robustness of the proposed method.

Table no. 2 - % RSD value for intra-day and inter-day precision respectively

Intra-day

No.	% RSD*	Regression	X Concentration	% Recovery		
				80	100	120
1	2.86	0.99795	211.18 ng	104	105	113
2	2.15	0.99723	200 ng	100	104	110
3	2.40	0.99842	207 ng	99	106	102

Inter-day

No.	% RSD*	Regression	X Concentration	% Recovery		
				80	100	120
1	2.86	0.99795	211.18 ng	104	105	113
2	2.13	0.99719	202 ng	99	98	107
3	2.38	0.99838	205 ng	101	100	97

***Results are mean of three determinations.**

Quntitative analysis of Embelin in *Embelia drupacea* (Dennst) M. R & S. M. Almeida

After chromatographic development the peak areas of the bands from sample were measured and the amount of embelin was determined from the respective calibration plots. The analytical procedure was repeated three times. Results were shown in Table No.3. Reported volume of embelin in *Embelia basaal* (previously known as *Embelia tsjeriam-cottam*) was 4.32% [4], in *Embelia ribes* it is 4.21 to 4.65 % [6], in marketed formulations of *Embelia ribes* - 2.19 to 2.20 % (w/w) [10] and in present research paper first time detected the volume of embelin in *Embelia*

drupacae found 2.05% (table no.3). Therefore *Embelia drupacae* could be use as substitute for Ayurvedic drug 'Vidanga' *Embelia ribes.*

Table no.3 Quantitative analysis of Embelin in *Embelia drupacea*

*Sample**	*Concentration*	*Embelin content w/w*
Embelia drupacea	*150 µg / spot*	*2.05 gm / 100 gm*

**Results are mean of three determinations.*

CONCLUSION

The proposed HPTLC method was developed and validated for simultaneous determination of Embelin. The method was found to be simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be successfully applied for routine quality control analysis and quantitative determination of Embelin from *Embelia drupacea* (Dennst) M. R & S. M. Almeida. However different pharmacognostic studies have to be carried out.

ACKNOWLEDGEMENT

The authors are thankful to Professor A. R. Kulkarni for valuable suggestions. They are also thankful to Dr. Sharad Medhe for kind help in the preparation of the manuscript and Anchrome lab. Ltd. Mulund, for providing facility to perform HPTLC analysis.

REFERENCE

1. Almeida, M. R., Almeida, S. M. Flora of Maharashtra.Vol. III (A/B) J. Bombay Nat. History. Soc. 90 : 426. (1993).
2. Kuruvilla, G., New quinine from *Maesa indica* (Roxb.) A. DC., (Myrsinaceae). *Indian Journal of chemistry. Vol. 49* B pp : 1637 -1641. (2010).
3. Mahadkar S.D., Jadhav Varsha. Traditional uses of some wild edible plants from Kolhapur district. *Life Sciences Leaflets* vol.5 P.19-26 (2013).
4. Mhaskar, M. Status of *Embelia ribes* Burm F.(Vidanga), an important medicinal species of commerce from Northen Western Ghats of India, *Current Science, vol.100*, no. 4, 547- 552,(2011).
5. Padma V., Evaluation of traditional anthelmintic herbs as substitutes for the endangered *Embelia ribes*, using Caenorhabditis elegans model. *Current science, Vol,105*: no. 11, (2013).
6. Quality standards of Indian Medicinal plants. Vol. IV. Published by Indian Council of Medical Research, New Delhi, P. 130-136. (2006).
7. REF- ICH Harmonized Tripartite Guideline, Validation of analytical procedures; Text and methodology Q2 (R1), Nov. 2005.
8. Syed, A., Preliminary Phytochemical investigation of *EMBELIA RIBES*, *Indian Journal of Research in Pharmacy and Chemistry. 3(2)*, page 389-391, (2011).
9. Sheth, A., The Herbs of Ayurveda, Vol. II, Ashok k Sheth Publisher Bhavnagar: 493 – 494. (2005).
10. Sudani R., Development and Validation of HPTLC method for Simultaneous Quantitation of Embelin and Assay of Marketed Formulation, *International Journal of Pharmaceutical and Biological Archives: 2 (2)* : 606-610, (2011).

***Corresponding Author:**
vpsathe14@gmail.com